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Silvestrol and Episilvestrol, Potential Anticancer Rocaglate Derivatives from *Aglaia silvestris*

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Two cytotoxic rocaglate derivatives possessing an unusual dioxanyloxy unit, silvestrol (**1**) and episilvestrol (**2**), were isolated from the fruits and twigs of *Aglaia silvestris* by bioassay-guided fractionation monitored with a human oral epidermoid carcinoma (KB) cell line. Additionally, two new baccharane-type triterpenoids, 17,24-epoxy-25-hydroxybaccharan-3-one (**3**) and 17,24-epoxy-25-hydroxy-3-oxobaccharan-21-oic acid (**4**), as well as eleven known compounds, 1β,6α-dihydroxy-4(15)-eudesmene (**5**), ferulic acid (**6**), grasshopper ketone (**7**), apigenin, cabraleone, chrysoeriol, 1β,4β-dihydroxy-6α,15α-epoxyeudesmane, 4-hydroxy-3-methoxyacetophenone, 4-hydroxyphenethyl alcohol, ocotillone, and β-sitosterol 3-*O*-β-D-glucopyranoside, were also isolated and characterized. The structures of compounds **1**−**4** were elucidated by spectroscopic studies and by chemical transformation. The absolute stereochemistry of silvestrol (**1**) was established by a X-ray diffraction study of its di-*p*-bromobenzoate derivative, and the structure of **3** was also confirmed by single-crystal X-ray diffraction. The isolates and chemical transformation products were evaluated for cytotoxicity against several human cancer cell lines, and silvestrol (**1**) and episilvestrol (**2**) exhibited potent in vitro cytotoxic activity. Silvestrol (**1**) was further evaluated in vivo in the hollow fiber test and in the murine P-388 leukemia model.

Introduction

The genus *Aglaia* (Meliaceae) consists of over 100 species, which are dioecious trees or shrubs with small fragrant flowers distributed in the tropical rain forests of Indonesia and Malaysia. Previous phytochemical studies on *Aglaia* species have resulted in the isolation of various triterpenoids (apotirucallane, cycloartane, dammarane, and tirucallane types), cyclopenta[*b*]benzofurans, cyclopenta[*b*]benzopyrans, bisamides, and lignans.[1] Among these previously known isolates, cyclopenta[*b*]benzofurans such as rocaglate and rocaglamide derivatives have attracted considerable interest due to their unusual carbon skeleton, and these compounds are confined to members of the genus *Aglaia*. Rocaglamide derivatives are known to be natural insecticides, which may be comparable in potency to the well-known natural insecticide, azadirachtin, from the neem tree, *Azadirachta indica* L.[2] Moreover, the antileukemic and/or cytotoxic activity of certain rocaglamide and rocaglate derivatives has been reported.[1a,3] Mechanistically, cyclopenta[*b*]benzofurans have been found to block protein synthesis and

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(1) (a) King, M. L.; Chiang, C.-C.; Ling, H.-C.; Fugita, E.; Ochiai, M.; McPhail, A. T. *J. Chem. Soc., Chem. Commun.* **1982**, 1150−1151. (b) Brader, G.; Vajrodaya, S.; Greger, H.; Bacher, M.; Kalchhauser, H.; Hofer, O. *J. Nat. Prod.* **1998**, *61*, 1482−1490. (c) Weber, S.; Puripattanavong, J.; Brecht, V.; Frahm, A. W. *J. Nat. Prod.* **2000**, *63*, 636−642. (d) Proksch, P.; Edrada, R.; Ebel, R.; Bohnenstengel, F. I.; Nugroho, B. W. *Curr. Org. Chem.* **2001**, *5*, 923−938. (e) Wang, B.-G.; Ebel, R.; Nugroho, B. W.; Prijono, D.; Frank, W.; Steube, K. G.; Hao, X.-J.; Proksch, P. *J. Nat. Prod.* **2001**, *64*, 1521−1526.

(2) (a) Janprasert, J.; Sarasook, C.; Sukumalanand, P.; Champagne, D. E.; Isman, M. B.; Wiriyachitra, P.; Towers, G. H. N. *Phytochemistry* **1993**, *32*, 67−69. (b) Ishibashi, F.; Satasook, C.; Isman, M. B.; Towers, G. H. N. *Phytochemistry* **1993**, *32*, 307−310. (c) Güssregen, B.; Fuhr, M.; Nugroho, B. W.; Wray, V.; Witte, L.; Proksch, P. *Z. Naturforsch.* **1997**, *52c*, 339−344. (d) Hiort, J.; Chaidir; Bohnenstengel, F. I.; Nugroho, B. W.; Schneider, C.; Wray, V.; Witte, L.; Hung, P. D.; Kiet, L. C.; Proksch, P. *J. Nat. Prod.* **1999**, *62*, 1632−1635. (e) Schneider, C.; Bohnenstengel, F. I.; Nugroho, B. W.; Wray, V.; Witte, L.; Hung, P. D.; Kiet, L. C.; Proksch, P. *Phytochemistry* **2000**, *54*, 731−736.

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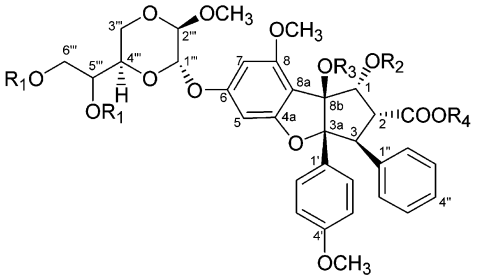
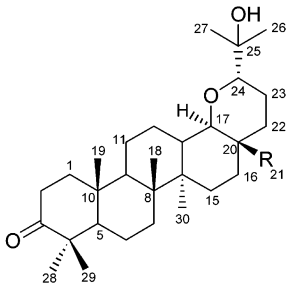
to induce growth arrest in the G_2/M-phase in certain tumor cell lines.[4] In addition, it was shown that rocaglamide and its derivatives represent highly potent and specific inhibitors of TNF-α or PMA-induced NF-κB activity in different mouse and human T cell lines.[5]

As a part of an ongoing collaborative search for novel anticancer agents from plant origin,[6] the chloroform extracts of the fruits and twigs of *Aglaia silvestris* (M. Roemer) Merrill (syn. *A. pyramidata* Hance) were found to exhibit significant cytotoxic activity against a number of human cancer cell lines. The chemical constituents of this plant have not been investigated previously. Bioassay-guided fractionation of these two crude extracts using the KB cell line to monitor fractionation resulted in the isolation of two new[7] cytotoxic rocaglate derivatives, silvestrol (**1**) and episilvestrol (**2**), two new triterpenoids (**3** and **4**), and eleven known compounds. Among these



1 (5‴*R*) $R_1 = R_2 = R_3 = H$ $R_4 = CH_3$
1a (5‴*R*) $R_1 = Ac$ $R_2 = R_3 = H$ $R_4 = CH_3$
1b (5‴*R*) $R_1 = COC_6H_5$ $R_2 = R_3 = H$ $R_4 = CH_3$
1c (5‴*R*) $R_1 = COC_6H_4(p)Br$ $R_2 = R_3 = H$ $R_4 = CH_3$
1d (5‴*R*) $R_1 = R_2 = COC_6H_4(p)Br$ $R_3 = H$ $R_4 = CH_3$
1e (5‴*R*) $R_1 = R_2 = R_3 = COC_6H_4(p)Br$ $R_4 = CH_3$
1f (5‴*R*) $R_1 = R_2 = R_3 = R_4 = H$
2 (5‴*S*) $R_1 = R_2 = R_3 = H$ $R_4 = CH_3$



3 R = CH_3
4 R = COOH
4a R = $COOCH_3$

isolates, silvestrol (**1**) exhibited potent in vitro cytotoxic activity comparable to that of the well-known anticancer drug, paclitaxel (Taxol), and it was further evaluated as a cytotoxic agent in both the hollow fiber assay and the P-388 lymphocytic leukemia system in mice. To confirm the structure of silvestrol (**1**), and in order to obtain a preliminary notion of structure-cytotoxic activity, several structurally modified derivatives (**1a**−**1f**) of silvestrol (**1**) were prepared. The absolute stereochemistry of silvestrol (**1**) was established by a single-crystal X-ray analysis of its di-*p*-bromobenzoate derivative (**1c**). We report herein the isolation and structure elucidation of compounds **1**−**4**, the cytotoxicity evaluation of the isolates and chemical transformation products against several human cancer cell lines, and the follow-up biological testing of **1** in two in vivo models.

Results and Discussion

Structure Elucidation of Silvestrol (1) and Episilvestrol (2). Silvestrol (**1**), obtained as amorphous powder, showed a sodiated molecular ion peak at *m*/*z* 677 $[M + Na]^+$ in the FABMS, and its molecular formula, $C_{34}H_{38}O_{13}$, was established by HRFABMS (*m*/*z* 677.2192 $[M + Na]^+$, calcd for $C_{34}H_{38}O_{13}Na$, 677.2210). IR absorptions implied the presence of hydroxy (3480 cm^{-1}) and ester carbonyl (1741 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) of **1** showed signals for three aromatic rings similar to those of methyl rocaglate,[2b,8] constituted by two *meta*-coupled aromatic protons at δ_H 6.27 (1H, d, $J = 1.5$ Hz, H-7) and 6.42 (1H, d, $J = 1.5$ Hz, H-5), a characteristic AA′BB′ system of a *p*-disubstituted benzene ring at δ_H 7.11 (2H, d, $J = 8.9$ Hz, H-2′ and H-6′) and 6.68 (2H, d, $J = 8.9$ Hz, H-3′ and H-5′), and the signals of a monosubstituted benzene ring at δ_H 7.05 (3H, m, H-3″, H-4″, and H-5″) and 6.85 (2H, m, H-2″ and H-6″). The ^1H NMR spectrum of **1** further exhibited signals at δ_H 5.04 (1H, d, $J = 6.6$ Hz, H-1), 3.89 (1H, dd, $J = 14.2$, 6.6 Hz, H-2), and 4.28 (1H, d, $J = 14.2$ Hz, H-3), typical for H-1, H-2, and H-3 of methyl rocaglate,[2b,8] respectively. Consistent with this ^1H NMR spectral data analysis, the ^{13}C NMR spectrum (Table 2) of **1** also displayed the signals for a tetrasubstituted, a disubstituted, and a monosubstituted benzene ring, as well as for a carboxylic group at δ_C 170.6, and two characteristic quaternary carbons at δ_C 101.9 and 93.4 of C-3a and C-8b of a rocaglate or rocaglamide derivative.[2b,8] Analysis of the remaining signals of the ^1H NMR spectrum indicated the presence of several oxygenated methine and methylene

(3) (a) Cui, B.; Chai, H.; Santisuk, T.; Reutrakul, V.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron* **1997**, *53*, 17625−17632. (b) Wu, T.-S.; Liou, M.-J.; Kuoh, C.-S.; Teng, C.-M.; Nagao, T.; Lee, K.-H. *J. Nat. Prod.* **1997**, *60*, 606−608. (c) Bohnenstengel, F. I.; Steube, K. G.; Meyer, C.; Nugroho, B. W.; Hung, P. D.; Kiet, L. C.; Proksch, P. *Z. Naturforsch.* **1999**, *54c*, 55−60. (d) Xu, Y.-J.; Wu, X.-H.; Tan, B. K. H.; Lai, Y.-H.; Vittal, J.-J.; Imiyabir, Z.; Madani, L.; Khozirah, K. S.; Goh, S. H. *J. Nat. Prod.* **2000**, *63*, 473−476. (e) Wang, S.-K.; Cheng, Y.-J.; Duh, C.-Y. *J. Nat. Prod.* **2001**, *64*, 92−94. (f) Wang, S.-K.; Duh, C.-Y. *Planta Med.* **2001**, *67*, 555−557.

(4) (a) Ohse, T.; Ohba, S.; Yamamoto, T.; Koyano, T.; Umezawa, K. *J. Nat. Prod.* **1996**, *59*, 650−652. (b) Lee, S. K.; Cui, B.; Mehta, R. R.; Kinghorn, A. D.; Pezzuto, J. M. *Chem.-Biol. Interact.* **1998**, *115*, 215−228. (c) Bohnenstengel, F. I.; Steube, K. G.; Meyer, C.; Quentmeier, H.; Nugroho, B. W.; Proksch, P. *Z. Naturforsch.* **1999**, *54c*, 1075−1083.

(5) Baumann, B.; Bohnenstengel, F.; Siegmund, D.; Wajant, H.; Weber, C.; Herr, I.; Debatin, K. M.; Proksch, P.; Wirth, T. *J. Biol. Chem.* **2002**, 277, 44791−44800.

(6) Kinghorn, A. D.; Farnsworth, N. R.; Soejarto, D. D.; Cordell, G. A.; Pezzuto, J. M.; Udeani, G. O.; Wani, M. C.; Wall, M. E.; Navarro, H. A.; Kramer, R. A.; Menendez, A. T.; Fairchild, C. R.; Lane, K. E.; Forenza, S.; Vyas, D. M.; Lam, K. S.; Shu, Y.-Z. *Pure Appl. Chem.* **1999**, *71*, 1611−1618.

(7) After assignment of the complete structures for compounds **1** and **2**, a literature survey indicated a patent application (Meurer-Grimes, B. M.; Yu, J.; Vairo, G. L. PCT Int. Appl. WO 2002002566, A1 20020110, 2002, 60 pp) on two similar rocaglate derivatives, in which the dioxanyloxy groups were assigned at C-8 instead of at C-6 in **1** and **2**, which were recently isolated from another *Aglaia* species, *A. leptantha*. The NMR data of silvestrol (**1**) and episilvestrol (**2**) are almost identical to those of the two analogues, so it is possible that the structures provided by those earlier authors were erroneous.

(8) Chaidir; Lin, W. H.; Ebel, R.; Edrada, R.; Wray, V.; Nimtz, M.; Sumaryono, W.; Proksch, P. *J. Nat. Prod.* **2001**, *64*, 1216−1220.

TABLE 1. ^{1}H NMR Spectral Data for Compounds 1, 2, and 1a−1f (500 MHz, $CDCl_3$)[a]

position	1	2	1a	1b	1c	1d	1e	1f
1	5.04, d (6.6)	5.04, d (6.6)	5.06, d (6.8)	4.98, d (6.7)	5.05, d (6.7)	6.29, d (7.1)	6.91, d (7.2)	5.01, d (6.5)
2	3.89, dd (14.2, 6.6)	3.90, dd (14.1, 6.8)	3.91, dd (14.2, 6.8)	3.82, dd (14.2, 6.7)	3.87, dd (14.2, 6.9)	4.02, dd (14.5, 7.0)	4.15, dd (14.7, 7.2)	3.86, dd (13.2, 6.5)
3	4.28, d (14.2)	4.28, d (14.1)	4.26, d (14.2)	4.19, d (14.2)	4.26, d (14.2)	4.32, d (14.5)	4.39, d (14.6)	4.26, d (13.2)
5	6.42, d (1.5)	6.45, d (1.6)	6.44, d (1.7)	6.44, d (1.8)	6.46, d (1.8)	6.52, d (1.8)	6.60, d (1.8)	6.40, d (1.6)
7	6.27, d (1.5)	6.29, d (1.6)	6.28, d (1.7)	6.31, d (1.8)	6.26, d (1.8)	6.03, d (1.8)	5.96, d (1.8)	6.23, d (1.6)
2′, 6′	7.11, d (8.9)	7.11, d (8.8)	7.10, d (8.9)	7.08, d (8.9)	7.08, d (8.9)	7.08, d (8.9)	7.08, d (8.9)	7.04−7.06, m
3′, 5′	6.68, d (8.9)	6.68, d (8.8)	6.67, d (8.9)	6.69, d (8.9)	6.68, d (8.9)	6.60, d (8.9)	6.52, d (8.9)	6.64, d (9.0)
2″, 6″	6.85, m	6.87, m	6.83, m	6.79, m	6.83, m	6.85, m	6.87, m	6.87, m
3″, 4″, 5″	7.05, m	7.06, m	7.05, m	7.04, m	7.05, m	7.06, m	7.07, m	7.04−7.06, m
1‴	5.22, br s	5.24, br s	5.38, br s	5.41, br s	5.37, br s	5.36, br s	5.33, br s	5.27, br s
2‴	4.56, br s	4.59, br s	4.61, br s	4.65, br s	4.63, br s	4.62, br s	4.60, br s	4.55, br s
3‴	3.53, br d (11.7)	3.77, dd (11.3, 2.1)	3.54, dd (11.4, 2.3)	3.68, dd (11.4, 2.3)	3.65, dd (11.3, 2.6)	3.65, dd (11.3, 2.5)	3.66, dd (11.4, 2.5)	3.49−3.51, m
	4.11, t (11.2)	4.02, t (11.3)	3.94, t (11.4)	4.09, t (11.3)	4.03, t (11.3)	4.05, t (11.3)	4.05, t (11.3)	4.07, t (11.1)
4‴	4.21, br d (11.0)	4.11, ddd (11.3, 5.9, 2.7)	4.38, dt (11.1, 3.0)	4.70, dt (11.1, 2.0)	4.60, dt (11.0, 2.8)	4.62, dt (11.0, 2.5)	4.63, dt (11.0, 2.5)	4.16, br d (11.1)
5‴	3.57, br s[c]	3.61, dd (10.7, 5.8)	5.12, ddd (9.5, 9.5, 3.6)	5.56, m	5.55, m	5.58, m	5.60, m	3.49−3.51, m
6‴	3.57, br s[c]	3.69−3.71, m	4.22, dd (11.3, 6.2)	4.49, m	4.48, m	4.50, m	4.58, m	3.49−3.51, m
		3.61−3.65, m	3.91, dd (11.3, 3.0)					
5‴-BB[b]								
2, 6				8.08, d (7.2)	7.59, d (8.5)	7.56, d (8.6)	7.51, d (8.6)	
3, 5				7.46, br t (7.6)	7.93, d (8.5)	7.85, d (8.6)	7.76, d (8.6)	
4				7.58, br t (7.4)				
6‴-BB[b]								
2, 6				7.70, d (7.2)	7.41, d (8.5)	7.41,d (8.7)	7.42,d (8.5)	
3, 5				7.17, br t (7.6)	7.63, d (8.5)	7.62, d (8.7)	7.60, d (8.5)	
4				7.07, m				
1-BB[b]								
2, 6						7.60, d (8.5)	7.53, d (8.5)	
3, 5						7.72, d (8.5)	7.73, d (8.5)	
8b-BB[b]								
2, 6							7.50,d (8.5)	
3, 5							7.89, d (8.5)	
$COOCH_3$-2	3.65, s	3.65, s	3.64, s	3.61, s	3.64, s	3.42, s	3.42, s	
OCH_3-8	3.86, s	3.87, s	3.89, s	3.79, s	3.82, s	3.00, s	3.00, s	3.78, s
OCH_3-4′	3.71, s	3.71, s	3.72, s	3.73, s	3.71, s	3. 62, s	3. 62, s	3.69, s
OCH_3-2‴	3.48, s	3.50, s	3.50, s	3.48, s	3.47, s	3.46, s	3.46, s	3.47, s
$COCH_3$-5‴			1.80, s					
$COCH_3$-6‴			2.14, s					

[a] TMS was used as the internal standard; chemical shifts are presented in parts per million. *J* values are given in Hz in parentheses. Assignments are based on ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. [b] Benzoyl or *p*-bromobenzoyl group. [c] The signals for H-5‴ and H_2-6‴ overlapped as a broad singlet.

TABLE 2. ^{13}C NMR Spectral Data for Compounds 1, 2, and 1a−1f (125 MHz, $CDCl_3$)[a]

position	1	2	1a	1b	1c	1d	1e	1f
1	79.7 d	79.6 d	79.7 d	79.7 d	79.9 d	78.9 d	77.6 d	79.6 d
2	50.3 d	50.2 d	50.4 d	50.5 d	50.5 d	50.0 d	49.2 d	50.1 d
3	55.0 d	55.1 d	54.9 d	55.2 d	55.0 d	55.0 d	54.9 d	55.0 d
3a	101.9 s	101.9 s	102.0 s	101.8 s	102.0 s	101.8 s	100.4 s	101.9 s
4a	160.6 s	160.5 s	160.6 s	160.6 s	160.8 s	161.0 s	161.6 s	160.7 s
5	92.9 d	92.9 d	93.3 d	92.6 d	93.4 d	92.4 d	92.3 d	92.6 d
6	160.0 s	159.9 s	159.8 s	160.3 s	160.2 s	160.5 s	160.9 s	160.0 s
7	93.9 d	94.3 d	93.3 d	93.8 d	93.6 d	93.4 d	92.6 d	93.8 d
8	157.1 s	157.1 s	157.0 s	157.1 s	157.1 s	157.1 s	157.4 s	157.3 s
8a	109.6 s	109.4 s	109.9 s	109.9 s	110.1 s	109.7 s	103.4 s	109.0 s
8b	93.4 s	93.4 s	93.6 s	93.5 s	93.7 s	93.7 s	93.6 s	93.3 s
1′	126.3 s	126.2 s	126.3 s	126.6 s	126.3 s	126.8 s	127.0 s	126.3 s
2′, 6′	129.0 d	128.9 d	129.1 d	129.3 d	129.3 d	130.2 d	130.9 d	128.9 d
3′, 5′	112.7 d	112.8 d	112.7 d	112.7 d	112.9 d	112.9 d	112.8 d	112.7 d
4′	158.8 s	158.8 s	158.7 s	158.9 s	158.9 s	158.6 s	158.5 s	158.8 s
1″	136.7 s	136.7 s	136.8 s	136.9 s	136.9 s	135.9 s	135.6 s	136.7 s
2″, 6″	127.8 d	127.8 d	127.7 d	128.0 d	128.1 d	128.0 d	127.9 d	127.9 d
3″, 5″	127.8 d	127.8 d	127.7 d	127.9 d	127.9 d	127.9 d	127.8 d	127.8 d
4″	126.6 d	126.7 d	126.6 d	126.8 d	126.8 d	127.0 d	127.1 d	126.7 d
1‴	94.0 d	93.7 d	93.8 d	94.3 d	94.5 d	94.5 d	94.4 d	94.4 d
2‴	95.2 d	95.2 d	95.3 d	95.6 d	95.6 d	95.6 d	95.5 d	95.2 d
3‴	59.0 d	59.7 d	58.8 t	59.0 t	58.9 t	58.6 t	58.5 t	59.0 d
4‴′	68.3 d	67.5 d	66.2 d	66.7 d	66.9 d	66.9 d	66.7 d	68.2 d
5‴	70.7 d	71.4 d	69.2 d	69.9 d	70.2 d	70.2 d	70.6 d	70.7 d
6‴	63.3 t	62.4 t	61.2 t	61.7 t	62.5 t	62.7 t	63.1 t	63.2 t
5‴-BB[b]								
1				129.3 s	128.2 s	128.2 s	128.3 s	
2, 6				130.1 d	132.2 d	131.8 d	131.5 d	
3, 5				128.8 d	131.6 d	131.4 d	131.2 d	
4				133.9 d	129.1 s	129.0 s	128.9 s	
7				166.0 s	165.4 s	165.4 s	165.2 s	
6‴-BB[b]								
1				129.2 s	128.1 s	128.0 s	128.0 s	
2, 6				129.7 d	131.9 d	131.5 d	131.3 d	
3, 5				128.4 d	131.3 d	131.1 d	131.1 d	
4				133.2 d	128.5 s	128.5 s	128.5 s	
7				166.0 s	165.4 s	165.0 s	165.2 s	
1-BB[b]								
1						128.2 s	128.0 s	
2, 6						128.7 s	128.6 s	
3, 5						131.8 d	131.8 d	
4						131.9 d	131.9 d	
7						168.7 s	168.8 s	
8b-BB[b]								
1							128.4 s	
2, 6							128.7 s	
3, 5							131.8 d	
4							131.9 d	
7							168.8 s	
$COCH_3$-2	170.6 s	170.8 s	170.3 s	170.6 s	170.6 s	170.8 s	171.0 s	173.2 s
	52.1 q	52.1 q	52.0 q	52.2 q	52.2 q	52.1 q	52.2 q	
OCH_3-8	55.9 q	55.9 q	56.0 q	56.0 q	56.0 q	56.2 q	56.4 q	55.9 q
OCH_3-4′	55.1 q	55.1 q	55.2 q	55.3 q	55.3 q	55.2 q	55.0 q	55.1 q
OCH_3-2‴	55.0 q	55.1 q	55.1 q	55.4 q	55.4 q	55.4 q	55.2 q	55.1 q
Ac-5‴			170.4 s					
			20.4 q					
Ac-6‴			170.7 s					
			20.9 q					

[a] TMS was used as internal standard; assignments are based on ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. [b] Benzoyl or *p*-bromobenzoyl group.

protons at δ_H 5.22 (1H, br s, H-1‴), 4.56 (1H, br s, H-2‴), 4.21 (1H, br d, J = 11.0 Hz, H-4‴), 4.11 (1H, t, J = 11.2 Hz, H-3‴a), 3.57 (3H, br s, H-5‴ and H_2-6‴), and 3.53 (1H, br d, J = 11.7, H-3‴b). Consistent with these ^{1}H NMR observations, the ^{13}C NMR spectrum of **1** displayed two doubly oxygenated methines in a downfield region (δ_C 94.0, C-1‴; 95.2, C-2‴), two oxygenated methines at δ_C 68.3 (C-4‴) and 70.7 (C-5‴), and two oxygenated methylenes at δ_C 59.0 (C-3‴) and 63.3 (C-6‴). These 1D NMR data, in combination with the observed 2D ^{1}H−^{1}H COSY, HMQC, and HMBC correlations (Figure 1), suggested the occurrence of an unusual [6-(1,2-dihydroxyethyl)-3-methoxy-1,4-dioxan-2yl]oxy moiety in the molecule of **1**. In the HMBC spectrum of **1**, the correlations from δ_H 5.04 (H-1), 3.89 (H-2), 4.28 (H-3), and 3.65 (−OCH_3) to δ_C 170.6 ($COOCH_3$) indicated the presence of a methyl ester functional group in the molecule of **1**, which could be located at C-2. The relative configuration of the rocaglate skeletal part of **1** was established primarily by analysis of the splitting patterns and

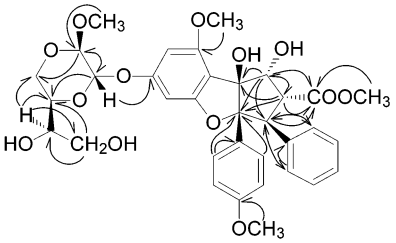


FIGURE 1. Selected HMBC correlations of silvestrol (**1**).

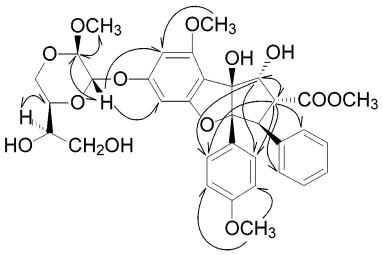


FIGURE 2. Selected NOESY correlations of silvestrol (**1**).

coupling constants of the ^{1}H NMR signals as well as the observed NOESY correlations (Figure 2). The vicinal coupling constant values between H-1 and H-2 ($J_{1,2}$ = 6.6 Hz) and between H-2 and H-3 ($J_{2,3}$ = 14.2 Hz) indicated the relative configuration of H-1, H-2, and H-3 to be α, α, and *β*, respectively, and rings B and C to be *cis*-fused.[3a,3b,8] These relative configurations were confirmed by a 2D NOESY experiment, wherein the correlations were observed from H-1 to H-2, H-2′, and H-6′, and from H-2 to H-1, H-2′, H-6′, H-2″, and H-6″. The remaining methoxy groups were assigned at C-8, C-4′, and C-2‴ based on the observed HMBC correlations from δ_H 3.86 (OCH_3-8) to δ_C 157.1 (C-8), δ_H 3.71 (OCH_3-4′) to δ_C 158.8 (C-4′), and δ_H 3.48 (OCH_3-2‴) to δ_C 95.2 (C-2‴). All of the above 1D and 2D NMR data suggested that compound **1** is a rocaglate derivative of aglafolin.[2b,3b] The substituted 1,4-dioxane moiety was placed at C-6 as a result of the observed correlations from δ_H 5.22 (H-1‴) to both δ_H 6.42 (H-5) and 6.27 (H-7) and from δ_H 3.86 (OCH_3-8) only to δ_H 6.27 (H-7) in the NOESY spectrum (Figure 2). Thus, silvestrol (**1**) was assigned as 6-*O*-demethyl-6-[6-(1,2-dihydroxyethyl)-3-methoxy-1,4-dioxan-2-yl]aglafolin.

The HRFABMS (*m*/*z* 677.2199 [M + Na]$^+$, calcd for $C_{34}H_{38}O_{13}Na$, 677.2210) of episilvestrol (**2**) supported a molecular formula of $C_{34}H_{38}O_{13}$, the same as that for silvestrol (**1**). The ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectral data of **2** were very close to those of **1** and suggested compound **2** to be also a rocaglate derivative possessing a 6-[(1,2-dihydroxyethyl)-3-methoxy-1,4-dioxan-2-yl]oxy unit, as in the case for **1**. Interpretation of the 2D NMR spectral data (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY) indicated the gross structure of compound **2** to be the same as **1**. The chemical shifts and coupling constants of rocaglate skeleton protons (H-1, H-2, and H-3) of compounds **1** and **2** were almost the same (Table 1), and the chemical shifts of all carbons of the rocaglate skeleton were also identical for compounds **1** and **2**. This suggested the difference between these two compounds was in the stereochemistry of the 1,4-dioxan portion of the molecules. Further comparison of the 1D NMR data disclosed that the resonance signals for H-1‴ and H-2‴ were broad singlets for both **1** and **2**, and the chemical shifts of H-1‴ and H-2‴ and C-1‴ and C-2‴ were closely comparable (Tables 1 and 2). However, the chemical shifts of H-3‴, H-4‴, H-5‴, and H-6‴ and C-3″, C-4‴, C-5‴, and C-6‴ were different (Tables 1 and 2), which suggested the stereochemistry of C-4‴ and/or C-5‴ varied between **1** and **2**. The splitting patterns and coupling constants of both H-3‴α (**1**: br d, 11.7 Hz. **2**: dd, 11.3, 2.1 Hz) and H-3‴*β* (**1**: t, 11.2 Hz. **2**: t, 11.3 Hz) were very similar and indicated that the orientation of H-4‴ in **2** was the same as that in **1**. Thus, the only difference between the molecules of **1** and **2** was the different configuration of C-5‴, which was supported by the different chemical shifts and splitting patterns of H-4‴, H-5‴, and H-6‴. In compound **1**, the signal of H-4‴ was displayed as a broad doublet (11.0 Hz), and the chemical shifts of the two protons of H-6‴ were the same and were overlapped with H-5‴ at δ_H 3.57 as a very broad singlet. However, in compound **2**, the signal of H-4‴ appeared as a double doublet of doublets (11.3, 5.9, 2.7 Hz), and the signals of the two protons of H-6‴ were separated from the H-5‴ signal (Supporting Information: Figures S4 and S5). Accordingly, compound **2** was assigned as the C-5‴ epimer of **1** and has been named episilvestrol.

Absolute Configuration of Silvestrol (1). The absolute configuration of rocaglamide-related compounds has so far been deduced only by chiroptical comparison with rocaglamide itself, whose stereochemistry was elucidated by enantioselective synthesis.[9] Recently, the absolute configurations of aglaroxin A (6-demethyl-10-hydroxy-11-methoxy-6,7-methylenedioxyrocaglamide) and cyclorocaglamide were determined as 1*R*, 2*R*, 3*S*, 3a*R*, and 8b*S* by comparison of their CD spectra with molecular dynamics simulation calculations.[10] The CD spectrum of **1** was very similar to the previously reported value for methyl rocaglate, with a prominent negative Cotton effect between 217 and 220 nm as the most characteristic feature. However, the relative stereochemistry from C-1‴, C-2‴, C-4‴, and C-5‴ of the dioxanyloxy unit to the rocaglate skeleton (C-1, C-2, C-3, C-3a, C-8b) was difficult to establish from the available NMR data. To confirm the structure of **1**, and to determine the relative and absolute stereochemistry, a single crystal of the 5‴,6‴di-*p*-bromobenzoate of **1** (**1c**) was prepared and purified from a CH_2Cl_2 and MeOH mixture. The X-ray crystallographic analysis of this heavy atom-containing analogue (**1c**) confirmed unambiguously the structure of silvestrol and permitted the absolute stereochemistry of this complex rocaglate derivative (**1**) to be determined as 1*R*, 2*R*, 3*S*, 3a*R*, 8b*S*, 1‴*S*, 2‴*R*, 4‴*R*, and 5‴*R* by the standard anomalous scattering method (Figure 3).

Structure Elucidation of Compounds 3 and 4. The HRFABMS of **3** provided an [M + Na]$^+$ peak at *m*/*z* 481.3634, indicating a molecular formula of $C_{30}H_{50}O_3$. Its IR spectrum showed hydroxyl (3460 cm^{-1}) and ketone (1704 cm^{-1}) functionalities. The ^{1}H NMR spectrum (Table

(9) (a) Trost, B. M.; Greenspan, P. D.; Yang, B. V.; Saulnier, M. G. *J. Am. Chem. Soc.* **1990**, *112*, 9022−9024. (b) Nugroho, B. W.; Edrada, R. A.; Güssregen, B.; Wray, V.; Witte, L.; Proksch, P. *Phytochemistry* **1997**, *44*, 1455−1461.

(10) (a) Dreyer, M.; Nugroho, B. W.; Bohnenstengel, F. I.; Ebel, R.; Wray, V.; Witte, L.; Bringmann, G. *J. Nat. Prod.* **2001**, *64*, 415−420. (b) Bringmann, G.; Muhlbacher, J.; Messer, K.; Dreyer, M.; Ebel, R.; Nugroho, B. W.; Wray, V.; Proksch, P. *J. Nat. Prod.* **2003**, *66*, 80−85.

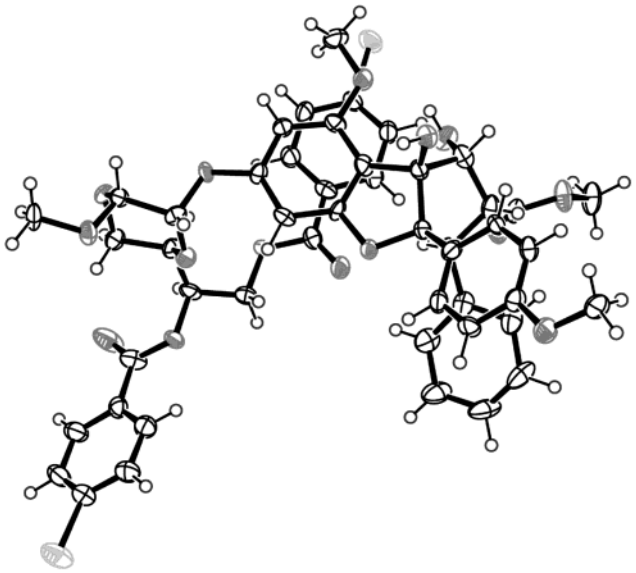


FIGURE 3. ORTEP drawing of the 5‴,6‴-di-*p*-bromobenzoate derivative of silvestrol (**1c**).

3) of **3** exhibited signals for eight tertiary methyl groups at δ_H 0.95 (s), 0.96 (s), 0.98 (s), 1.03 (s), 1.06 (s), 1.08 (s), 1.16 (s), and 1.19 (s) and two characteristic oxygen-bearing methine groups at δ_H 3.45 (d, J = 10.7 Hz, H-17) and 3.58 (dd, J = 7.4, 6.2 Hz, H-24). The ^{13}C NMR and DEPT spectra (Table 3) exhibited 30 signals (eight CH_3, ten CH_2, five CH, and seven C) including a nonconjugated ketone (δ_C 218.1), an oxygenated quaternary carbon (δ_C 74.1, C-25), and two oxygenated methine carbons (δ_C 76.0, C-17, and 78.2, C-24). The nonconjugated ketone was placed at C-3 based on the observed HMBC correlations from the proton signals of H_2-1, H_2-2, CH_3-28, and CH_3-29 to this carbon signal. The signals of two methyl groups at δ_H 1.16 (CH_3-26) and 1.19 (CH_3-27) showed HMBC correlations to the oxygenated quaternary carbon (δ_C 74.1, C-25) and the oxygenated methine (δ_C 78.2, C-24), respectively, and indicated that a 2-hydroxyisopropyl group was attached at C-24 in compound **3**. This attachment was confirmed by HMBC correlations from δ_H 3.58 (H-24) to δ_C 74.1 (C-25), 25.8 (C-26), 26.8 (C-27), 76.0 (C-17), 19.6 (C-23), and 35.9 (C-22). The observed HMBC correlations from δ_H 3.45 (H-17) to δ_C 78.2 (C-24), 35.3 (C-16), 35.9 (C-22), and 19.4 (C-21) clearly indicated the presence of an 17,24-epoxy functionality. The relative stereochemistry at C-24 was determined by the NOESY spectrum, in which a correlation was observed between H-24 and CH_3-21. The oxymethine signal at δ_H 3.45 (H-17) displayed significant NOESY correlations with CH_3-30, CH_3-26, and CH_3-27, and these observations allowed the relative configuration of C-17 to be determined. Thus, compound **3** was assigned as 17,24-epoxy-25-hydroxybaccharan-3-one. The baccharane-type triterpenoids were first isolated from *Baccharis halimifolia* L. in 1970.[11] To confirm the presence of the 17,24-epoxy functionality in the molecule of **3**, an X-ray analysis was performed on a single crystal obtained from a $CHCl_3$−MeOH mixture (Figure 4).

The HRESIMS of **4** provided an $[M + Na]^+$ peak at m/z 511.3400, indicating the molecular formula of $C_{30}H_{48}O_5$ (calcd for $C_{30}H_{48}O_5Na$, 511.3399). The ^{1}H and ^{13}C NMR spectra (Table 3) of **4** were very similar to those of **3**, except that a carboxylic acid group resonance at δ_C 176.7 in **4** replaced a methyl group signal in **3**. In the HMBC spectrum of **4**, the correlation from δ_H 4.05 (H-17) to the carbonyl carbon δ_C 176.7 (C-21) was observed. This indicated the carboxylic acid group in **4** was located at C-20. Thus, compound **4** was assigned as 17,24-epoxy-

(11) Anthonsen, T.; Bruun, T.; Hemmer, E.; Holme, D.; Lamvik, A.; Sunde, E.; Soerensen, N. A. *Acta Chem. Scand.* **1970**, *24*, 2479−2488.

TABLE 3. NMR Spectral Data for Compounds 3, 4, and 4a (360/90 MHz, $CDCl_3$)[a]

position	3		4		4a	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.42[b], 1.92[b]	39.7 t	1.40[b], 2.01[b]	39.9 t	1.40[b], 1.95[b]	39.7 t
2	2.43[b], 2.50[b]	34.1 t	2.39[b], 2.55[b]	34.3 t	2.39[b], 2.50[b]	36.9 t
3		218.1 s		218.2 s		218.1 s
4		47.3 s		47.6 s		47.4 s
5	1.32[b]	55.0 d	1.35[b]	55.2 d	1.35[b]	55.0 d
6	1.47[b], 1.76[b]	19.7 t	1.45[b], 1.49[b]	19.7 t	1.45[b], 1.50[b]	19.6 t
7	1.40[b]	32.8 t	1.40[b]	33.0 t	2.45[b]	34.1 t
8		40.8 s		42.8 s		47.3 s
9	1.43[b]	49.9 d	1.47[b]	50.0 d	1.45[b]	50.1 d
10		36.9 s		37.1 s		38.1 s
11	1.32[b], 1.55[b]	21.0 t	1.35[b], 1.59[b]	21.0 t	1.40[b], 1.60[b]	21.0 t
12	1.11[b], 1.96[b]	23.9 t	1.15[b], 2.06[b]	24.1 t	1.15[b], 2.05[b]	24.0 t
13	1.74[b]	37.5 d	1.80[b]	40.4 d	1.85[b]	40.7 d
14		42.7 s		40.9 s		42.3 s
15	0.97[b], 1.64[b]	26.4 t	1.23[b], 1.51[b]	28.4 t	1.10[b], 1.51[b]	28.0 t
16	1.34[b]	35.3 t	1.27[b], 2.22[b]	30.8 t	1.35[b], 2.30[b]	32.4 t
17	3.45, d (10.7)	76.0 d	4.05, d (11.3)	76.2 d	3.65, d (11.0)	75.7 d
18	1.06, s	15.5 q	0.98, s	15.7 q	1.02, s	15.5 q
19	0.95, s	16.0 q	0.94, s	16.2 q	0.95, s	16.0 q
20		33.9 s		47.4 s		47.3 s
21	0.98, s	19.4 q		176.7 s		175.6 s
22	1.38[b], 1.54[b]	35.9 t	1.79[b], 1.98[b]	32.1 t	1.69[b], 1.90[b]	32.8 t
23	1.45[b], 1.75[b]	19.6 t	1.90[b], 2.02[b]	20.4 t	1.85[b], 1.95[b]	20.7 t
24	3.58, dd (7.4, 6.2)	78.2 d	3.71, t (5.2)	79.7 d	3.55, t (5.8)	77.8 d
25		74.1 s		75.5 s		74.8 s
26	1.16, s	25.8 q	1.32, s	27.3 q	1.17, s	26.9 q
27	1.19, s	26.8 q	1.23, s	28.2 q	1.23, s	27.1 q
28	1.08, s	26.6 q	1.07, s	26.8 q	1.07, s	26.6 q
29	1.03, s	21.0 q	1.03, s	21.2 q	1.03, s	21.0 q
30	0.96, s	15.0 q	0.97, s	15.3 q	0.96, s	15.2 q
OMe					3.73, s	51.6 q

[a] TMS was used as internal standard; chemical shifts are shown in the δ scale with J values (Hz) in parentheses. Assignments are based on ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. [b] Multiplicity patterns were unclear due to signal overlapping.

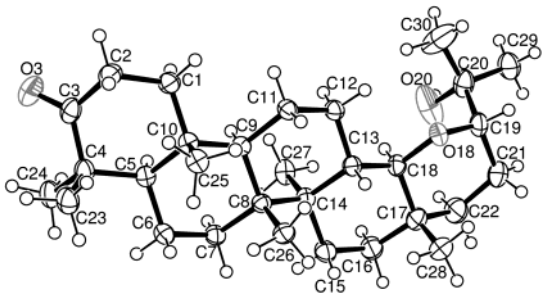
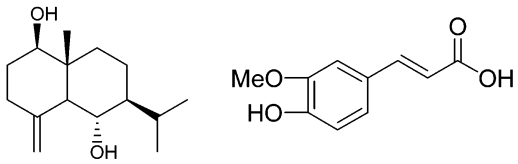


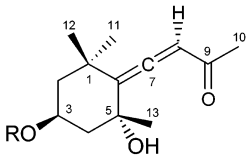
FIGURE 4. ORTEP drawing of compound **3** (Numbering does not follow that of Chemical Abstracts).

25-hydroxy-3-oxobaccharan-21-oic acid. The relative stereochemistry of C-17 was determined by the NOESY spectrum, in which NOE enhancements were observed from H-17 to CH_3-30, CH_3-26, and CH_3-27, and these observations allowed for the determination of the α-orientation of H-17. The methylation product **4a** was obtained by treating compound **4** with excess CH_2N_2. In the NOESY spectrum of **4a**, the methoxy signal of the methyl ester group at δ_H 3.73 (COOMe) displayed a significant NOESY correlation with H-13, which suggested the C and D rings in **4** were *trans*-fused as in the case for **3**.

In addition to above-determined four new compounds (**1**−**4**), eleven known compounds, 1*β*,6α-dihydroxy-4(15)-eudesmene (**5**),[12] ferulic acid (**6**),[13] grasshopper ketone (**7**),[14] apigenin,[15] cabraleone,[16] chrysoeriol,[17] 1*β*,4*β*-dihydroxy-6α,15α-epoxyeudesmane,[18] 4-hydroxy-3-methoxyacetophenone,[19] 4-hydroxyphenethyl alcohol (tyrosol),[20] ocotillone,[21] and *β*-sitosterol 3-*O*-*β*-D-glucopyranoside, were isolated from the $CHCl_3$-soluble extracts (Experimental Section) of the separate extracts of the fruits and twigs of *A. silvestris*. The structures of these known



5 **6**



7 R = H
7r R = (*R*)-MTPA
7s R = (*S*)-MTPA

compounds were identified by comparing their physical and spectroscopic data ($[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, DEPT, 2D NMR, and MS) with those of published values or by comparing with an authentic sample (*β*-sitosterol 3-*O*-*β*-D-glucopyranoside) directly. Grasshopper ketone (**7**) possesses an unusual allenic group, and its structure including absolute stereochemistry was previously confirmed by semisynthesis,[22] total synthesis,[23] and X-ray analysis.[24] In the present study, this compound was treated with (*R*)- and (*S*)-MTPA-Cl in deuterated pyridine directly in NMR tubes,[25] to afford the (*S*)- and (*R*)-MTPA ester, respectively. The ^{1}H NMR spectral data obtained for the (*R*)- and (*S*)-MTPA esters of **7** (Supporting Information) enabled the absolute configuration of C-5 to be confirmed as *R*, which is the same as in the previous assignments.[22−24] The NMR data of compounds **7**, **7r**, and **7s** were assigned (Supporting Information) by analysis of their 2D NMR spectral data.

TABLE 4. Cytotoxic Activity of Compounds 1, 1a−1c, 1f, 2, 5, and 6[a]

compd	cell line[b] Lu1	LNCaP	MCF-7	HUVEC
1	1.2	1.5	1.5	4.6
1a	4.1	27.1	2.7	135.5
1b	2088.2	46.4	104.4	58.0
1c	491.2	196.5	98.2	392.9
1f	796.9	1171.9	1250.0	3750.0
2	3.8	3.8	5.5	15.3
5	1680.7	3361.3	420.2	7563.0
6	5670.1	46 391.8	17 010.3	>103 092.8
paclitaxel[c]	2.3	4.7	0.7	105.5
camptothecin[c]	28.7	28.7	28.7	258.6

[a] New triterpenoids **3** and **4** and all other known compounds and chemical modification products obtained in the present study were considered to be inactive, since their ED_{50} values were > 5 *μ*g/mL against the tested cell lines. [b] Results are expressed as ED_{50} values (nM). Key to cell lines used: Lu1 = human lung cancer; LNCaP = hormone-dependent human prostate cancer; MCF-7 = human breast cancer; HUVEC = human umbilical vein endothelial cells. [c] Used as positive control substances.

Biological Activity. The isolates and chemical transformation products obtained in the present investigation were evaluated for their cytotoxic activity against several human cancer cell lines (Table 4).[26] Among the four new compounds (**1**−**4**), the rocaglate derivatives, silvestrol (**1**) and episilvestrol (**2**), were found to be significantly active principles, while the triterpenoids **3** and **4** were indicated to be inactive (ED_{50} > 5 *μ*g/mL). In the tested cell lines, the activities of silvestrol (**1**) were approximately 3 times more potent than those of episilvestrol (**2**), although compound **2** also showed very strong activity comparable to paclitaxel (Taxol) (Table 4). To confirm the structure and improve the activity, six chemical transformation products (**1a**−**1f**; NMR data, see Tables 1 and 2; the preparation procedures and physical and other spectroscopic data of these derivatives, see Supporting Information) of silvestrol were prepared. However, all of these semisynthetic products lost potency in comparison to silvestrol (**1**), and some of these were inactive (Table 4). Among the eleven known compounds obtained in this study, only

(12) Kitajima, J.; Suzuki, N.; Satoh, M.; Watanabe, M. *Phytochemistry* **2002**, *59*, 811−815.
(13) Lee, H.-S.; Beon, M.-S.; Kim, M.-K. *J. Agric. Food Chem.* **2001**, *49*, 4656−4661.
(14) (a) Meinwald, J.; Erickson, K.; Hartshorn, M.; Meinwald, Y. C.; Eisner, T. *Tetrahedron Lett.* **1968**, 2959−2962. (b) Hashimoto, T.; Tori, M.; Asakawa, Y. *Phytochemistry* **1991**, *30*, 2927−2931.
(15) Abegaz, B. M.; Ngadjui, B. T.; Dongo, E.; Tamboue, H. *Phytochemistry* **1998**, *49*, 1147−1150.
(16) Waterman, P. G.; Ampofo, S. *Phytochemistry* **1985**, *24*, 2925−2928.
(17) Susumu, K.; Michio, T. *Chem. Pharm. Bull.* **1992**, *40*, 249−251.
(18) Fang, N.; Yu, S.; Mabry, T. J.; Abboud, K. A.; Simonsen, S. H. *Phytochemistry* **1988**, *27*, 3187−3196.
(19) Crestini, C.; D'Auria, M. *Tetrahedron* **1997**, *53*, 7877−7888.
(20) Rasser, F.; Anke, T.; Sterner, O. *Phytochemistry* **2000**, *54*, 511−516.
(21) Govindachari, T. R.; Suresh, G.; Kumari, G. N. K. *Phytochemistry* **1994**, *37*, 1127−1129.
(22) DeVille, T. E.; Hursthouse, M. B.; Russell, S. W.; Weedon, B. C. L. *Chem. Commun.* **1969**, 1311.
(23) Mori, K. *Tetrahedron* **1974**, *30*, 1065−1072.
(24) Low, J. N.; Tollin, P.; Fleischer, E. B. *Acta Crystallogr.* **1987**, *C43*, 571−572.
(25) (a) Su, B.-N.; Park, E. J.; Mbwambo, Z. H.; Santarsiero, B. D.; Mesecar, A. D.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2002**, *65*, 1278−1282. (b) Su, B.-N.; Park, E. J.; Santarsiero, B. D.; Mesecar, A. D.; Soejarto, D. D.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *Phytochemistry* **2003**, *64*, 293−302.
(26) (a) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M. *J. Nat. Prod.* **1993**, *56*, 30−38. (b) Seo, E.-K.; Kim, N.-C.; Mi, Q.; Chai, H.; Wall, M. E.; Wani, M. C.; Navarro, H. A.; Burgess, J. P.; Graham, J. G.; Cabieses, F.; Tan, G. T.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2001**, *64*, 1483−1485.

1β,6α-dihydroxy-4(15)-eudesmene (**5**) and ferulic acid (**6**) exhibited evident cytotoxic effects (Table 4).

Silvestrol (**1**) was further evaluated with an in vivo murine hollow fiber test. The hollow fiber assay, developed at the U.S. National Cancer Institute (NCI), is a screening tool for assessing the potential anticancer activity of compounds against human tumor cells cultivated in hollow fibers and implanted intraperitoneally and subcutaneously in mice.[26b,27,28] After treatment by the ip route, fiber cultures were collected, and the viable cell mass was determined. At the doses (0.625, 1.25, 2.5, and 5 mg/kg body weight) tested, silvestrol (**1**) showed 11.6−63.2% and 0−26.8% inhibition of the growth of KB cells implanted at the intraperitoneal (ip) and subcutaneous (sc) compartments of mice, respectively. Also at these doses, inhibition of LNCaP cells was observed both at the ip and sc sites (14.9−82.5% and 12.4−15.7%, respectively). The growth of Col2 cells was inhibited by 20.5−76.9% and 4.7−23.4% at the ip and sc sites, respectively. No significant weight loss was observed in the test mice in all cases (Supporting Information, Figure S13).

Silvestrol (**1**) was also tested in two different versions of the P388 murine leukemia model.[29] More specifically, silvestrol was active at its maximum tolerated dose of 2.5 mg/kg/inj, when given by intraperitoneal injection daily for five consecutive days (qd × 5) in the ip P388 model. Here, a maximum increase in lifespan corresponding to a T/C of 150% was achieved. Although silvestrol was inactive (T/C = 100%) in the iv P388 leukemia model when administered by either the iv or ip route using a daily times 5 schedule (qd × 5), the compound was active (T/C = 129%) in this same tumor model when injected iv on a twice daily schedule (2qd × 5) using the same cumulative dose (2 mg/kg/day). This compound and its analogues are therefore worthy of further investigation for their potential as new cancer chemotherapeutic agents.

Experimental Section

Plant Material. The fruits and twigs of *A. silvestris* were individually collected by L.B.S.K. and S.R. at Timpah village, Kapuas Regency, Central Kalimantan, Indonesia, in August 2000. The voucher specimens (A5056 for fruits and A5054 for twigs) have been deposited at the Herbarium of the Field Museum of National History, Chicago, IL.

Extraction and Isolation of the Fruits. The dried fruits (1.0 kg) of *A. silvestris* were extracted 3 times with MeOH (3 × 2.5 L) overnight at room temperature. The solvent was evaporated in vacuo to afford a concentrated MeOH extract, which was then diluted with H_2O (0.9 L) to give an aqueous MeOH solution (1.0 L). The aqueous solution was partitioned in turn with *n*-hexane (2 × 1.0 L) and $CHCl_3$ (3 × 1.5 L), to afford dried *n*-hexane- (D001, 21.0 g), $CHCl_3$- (D002, 10.0 g), and H_2O-soluble (40.5 g) residues. The $CHCl_3$-soluble extract exhibited significant cytotoxicity against several human cancer cell lines, while the *n*-hexane and aqueous fractions were inactive. Accordingly, the $CHCl_3$ extract (D002, 10.0 g; KB, ED_{50} < 0.16 μg/mL) was subjected to silica gel column chromatography (6 × 40 cm, 70−230 mesh silica gel) and eluted with pure $CHCl_3$ initially, then with a gradient mixture of $CHCl_3$−MeOH (from 99:1 to 5:1), to afford eight fractions (F01−F08). These fractions were again evaluated in the KB cell line, and the ED_{50} (μg/mL) values were >20, 14.1, 4.0, 18.5, <0.16, <0.16, >20, and >20, respectively.

Fraction F02 (1.2 g; KB, ED_{50} 14.1 μg/mL), eluted with $CHCl_3$−MeOH (99:1), was subjected to further silica gel column chromatography (2.5 × 35 cm), eluted with $CHCl_3$−acetone (49:1), to give pure compounds **3** (8.0 mg) and **4** (4.0 mg). Fraction F03 (1.5 g; KB, ED_{50} 4.0 μg/mL), eluted with $CHCl_3$−MeOH (50:1), was subjected to passage over a silica gel column (2.5 × 35 cm), eluted with $CHCl_3$−EtOAc−MeOH (80:15:5), to give cabraleone (8.0 mg) and ocotillone (5.0 mg).

Fraction F05 (1.0 g; KB, ED_{50} < 0.16 μg/mL), one of the two most active fractions, eluted with $CHCl_3$−MeOH (20:1), was chromatographed over a Sephadex LH-20 column (3 × 30 cm), eluted with CHCl−MeOH (1:1), and afforded six fractions (F0501−F0506). Fraction F0502 (450 mg) was further purified over a silica gel column (2.5 × 35 cm), using $CHCl_3$−EtOAc−MeOH (75:25:5), to give pure silvestrol (**1**, 100 mg). Fraction F06 (1.2 g; KB, ED_{50} < 0.16 μg/mL), the second most active fraction, eluted with $CHCl_3$−MeOH (10:1), was chromatographed over a Sephadex LH-20 column (3 × 30 cm), eluted with $CHCl_3$−MeOH (1:1), and afforded six fractions (F0601−F0606). Fraction F0604 (30 mg) was subjected to silica gel column chromatography (2.5 × 35 cm), using $CHCl_3$−EtOAc−MeOH (75:25:5) as eluent, to give 1β,6α-dihydroxy-4(15)-eudesmene (**5**, 5.0 mg), 4-hydroxy-3-methoxyacetophenone (4.0 mg), and 4-hydroxyphenethyl alcohol (8 mg).

Extraction and Isolation of the Twigs. The dried and milled twigs (704 g) of *A. silvestris* were extracted and partitioned using the same method as described above for the fruits, to afford dried *n*-hexane- (5.8 g), $CHCl_3$- (12.0 g), and H_2O-soluble (20.5 g) residues. The bioassay test results indicated that only the $CHCl_3$-soluble extract showed significant cytotoxicity activity against the KB cell line (ED_{50} 0.1 μg/mL). Therefore, this extract was chromatographed over a silica gel column (6 × 40 cm, 70−230 mesh silica gel), eluting with pure $CHCl_3$ and then a gradient mixture of $CHCl_3$−MeOH (from 99:1 to 5:1), to afford nine fractions (F01−F09). These fractions were again evaluated in KB cell line, and the ED_{50} (μg/mL) values were >20, 8.9, 7.0, 5.2, <0.16, 1.5, 1.4, 2.6, and 1.4, respectively.

The most active fraction, F05 (0.98 g; KB, ED_{50} < 0.16 μg/mL), eluted with $CHCl_3$−MeOH (20:1), was selected for further detailed fractionation. This fraction was further purified over a Sephadex LH-20 column (3 × 30 cm), eluted with pure MeOH, and afforded 9 fractions (F0501−F0509). Fraction F0504 was chromatographed over a silica gel column (3.8 × 45 cm), eluted with *n*-hexanes−EtOAc−MeOH (50:50:1, 40:40:1, 30:30:1, 20:20:1, 10:10:1, and 5:5:1), to give twelve fractions (F050401−F050412). Fraction F050405 was then passed over a C_{18} reversed-phase silica gel column (3 × 30 cm), with MeOH−H_2O (40:60 and 60:40) as eluent, and yielded 1β,4β-dihydroxy-6α,15α-epoxyeudesmane (4.5 mg). Fractions F050407 and F050410 were separately purified by preparative TLC (20 × 20 cm, 500 μm), developed with $CHCl_3$−MeOH (11:1) and $CHCl_3$−MeOH (12:1), to give grasshopper ketone (**7**, 8.5 mg, R_f = 0.70) and episilvestrol (**2**, 4.5 mg, R_f = 0.48), respectively. Fraction F050411 was purified over a silica gel column (2.5 × 35 cm), with $CHCl_3$−MeOH (25:1) as solvent system, and afforded silvestrol (**1**, 60 mg). Ferulic acid (**6**, 2.4 mg, R_f = 0.54) was obtained from fraction F0508 by preparative TLC (20 × 20 cm, 1000 μm), developed with $CHCl_3$−

(27) (a) Casciari, J. J.; Hollingshead, M. G.; Alley, M. C.; Mayo, J. G.; Malspeis, L.; Miyauchi, S.; Grever, M. R.; Weinstein, J. N. *J. Natl. Cancer Inst.* **1994**, *86*, 1846−1852. (b) Hollingshead, M. G.; Alley, M. C.; Camalier, R. F.; Abbott, B. J.; Mayo, J. G.; Malspeis, L.; Grever, M. R. *Life Sci.* **1995**, *57*, 131−141. (c) Hall, L.-A. M.; Krauthauser, C. M.; Wexler, R. S.; Hollingshead, M. G.; Slee, A. M.; Kerr, J. S. *Anticancer Res.* **2000**, *20* (2A), 903−911.

(28) (a) Mi, Q.; Lantvit, D.; Reyes-Lim, E.; Chai, H.; Zhao, W.; Lee, I.-S.; Peraza-Sánchez, S.; Ngassapa, O.; Kardono, L. B. S.; Riswan, S.; Hollingshead, M. G.; Mayo, J. G.; Farnsworth, N. R.; Cordell, G. A.; Kinghorn, A. D.; Pezzuto, J. M. *J. Nat. Prod.* **2002**, *65*, 842−850. (b) Mi, Q.; Cui, B.; Chavez, D.; Chai, H.; Zhu, H.; Cordell, G. A.; Hedayat, S.; Kinghorn, A. D.; Pezzuto, J. M. *Anticancer Res.* **2002**, *22*, 1385−1397.

(29) Rose, W. C.; Schurig, J. E.; Meeker, J. B. *Anticancer Res.* **1988**, *8*, 355−368.

MeOH (12:1). Fraction F0509 was chromatographed over a silica gel column (1.5 × 15 cm), using the solvent system $CHCl_3$−MeOH (18:1), to give apigenin (0.9 mg) and chrysoeriol (1.7 mg). β-Sitosterol 3-*O*-β-D-glucopyranoside (28 mg) was obtained as a white amorphous powder from a solution of $CHCl_3$−MeOH (~5:1) of fraction F07, which was collected from the initial silica gel column chromatography by eluting with $CHCl_3$−MeOH (10:1).

Data for Silvestrol (1): white amorphous powder; mp 119−123 °C; $[\alpha]^{20}{}_D$ −137.0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.24), 273 (3.32) nm; IR (film) ν_{max} 3480, 1741, 1612, 1514, 1453, 1251, 1217, 1169, 1133, 1063, 755 cm^{-1}; CD (*c* 0.152 mM; MeOH) nm $\Delta\epsilon_{217}$ −17.96, $\Delta\epsilon_{253}$ −0.63, $\Delta\epsilon_{301}$ +0.15, $\Delta\epsilon_{275}$ −1.25; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; FABMS *m*/*z* 677 $[M + Na]^+$ (3), 498 (2), 475 (3), 325 (18), 199 (18), 176 (100), 91 (22); HRFABMS *m*/*z* 677.2192 $[M + Na]^+$ (calcd for $C_{34}H_{38}O_{13}Na$, 677.2210).

Data for Episilvestrol (2): yellowish gum; $[\alpha]^{20}{}_D$ −94.5° (*c* 0.43, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 223 (4.18), 274 (3.30) nm; IR (film) ν_{max} 3467, 1741, 1613, 1514, 1452, 1168, 1063, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; FABMS *m*/*z* 677 $[M + Na]^+$ (10), 625 (10), 498 (20), 475 (30), 348 (40), 325 (95), 199 (100), 172 (100), 91 (100); HRFABMS *m*/*z* 677.2199 $[M + Na]^+$ (calcd for $C_{34}H_{38}O_{13}Na$, 677.2210].

Data for 17,24-Epoxy-25-hydroxybaccharan-3-one (3): colorless needle crystals; mp 180−182 °C; $[\alpha]^{20}{}_D$ +16.8° (*c* 0.35, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 213 (3.56) nm; IR (film) ν_{max} 3460, 1704, 1068, 734 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; FABMS *m*/*z* 481 $[M + Na]^+$; HRFABMS *m*/*z* 481.3634 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_3Na$, 481.3658).

Data for 17,24-Epoxy-25-hydroxy-3-oxobaccharan-21-oic Acid (4): amorphous solid; mp 234−237 °C; $[\alpha]^{20}{}_D$ +32.6° (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 211 (3.35) nm; IR (film) ν_{max} 3458, 1725, 1712, 1131, 1064 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; EIMS *m*/*z* 470 $[M - H_2O]^+$ (2), 429 $[M - C_3H_7O]^+$ (100), 411 (23), 205 (25), 177 (11), 147 (11), 119 (12), 81 (80); HRESIMS *m*/*z* 511.3400 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_5Na$, 511.3399).

Bioassay Evaluation Procedures. The cytotoxic activity of the isolates and chemical transformation products was evaluated against a panel of human cancer cell lines (Table 4), according to established protocols.[26]

In Vivo Evaluation of Compound 1. Silvestrol (**1**) was evaluated for its biological potential in two in vivo test systems, namely, the murine hollow fiber[26b,27,28] and P-388 leukemia[29] models as described previously. In brief, P388 leukemia tumors were propagated in female DBA/2 mice (5−6 weeks of age) obtained from Harlan Sprague−Dawley Co. (Indianapolis, IN) and maintained in an ammonia-free environment in a defined and pathogen-free colony. Animals were quarantined for a week prior to use for tumor propagation and drug efficacy testing. They were fed food and water ad libitum. Antitumor activity in the P388 model was evaluated in terms of increases in lifespan reflected by the relative median survival time (MST) of treated (T) versus control (C) groups (i.e., %T/C values). The activity criterion for increased lifespan was a T/C of ≥125%. The dose of a compound that yielded the maximum therapeutic effect was termed the optimal dose (OD). Groups of mice with more than 33% death attributable to drug toxicity were considered to have had excessively toxic treatments, and their data were not used in the evaluation of antitumor activity.

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Supporting Information Available: ^1H NMR spectra for compounds **1**, **2**, **4**, and **7**; ^{13}C NMR spectra of compounds **1**, **4**, and **7** in $CDCl_3$; and ^1H NMR and $^1H-^1H$ COSY spectra of the (*R*)- and (*S*)-MTPA esters of compound **7** in pyridine-d_5 (obtained by running reaction NMR tubes directly), a figure of the effects of silvestrol (**1**) on the growth of KB, LNCaP, and Col2 cells implanted at the ip and the sc compartments of NCr *nu*/*nu* mice in the in vivo hollow fiber assay; the preparation procedures and physical data of **1a**−**1f** and **4a**; the X-ray crystallography data for **1c** and **3**; the NMR data assignment of compounds **7**, **7r**, and **7s**; and the general procedures (total 19 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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